Skeletal Responses to Long-Duration Simulated Weightlessness in Rats

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Abstract

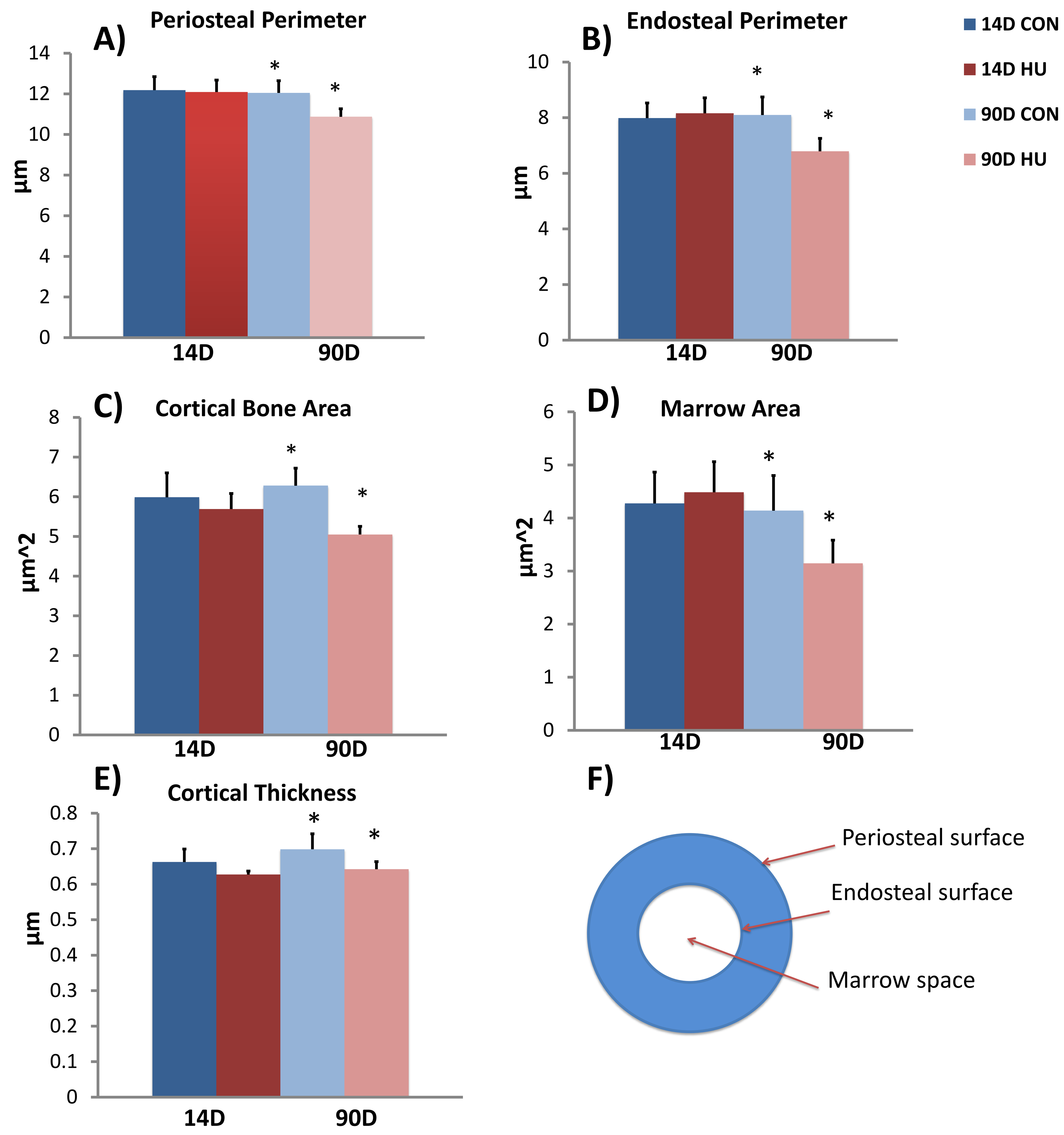
Damaging effects due to spaceflight and long-duration weightlessness are seen in the musculoskeletal system, specifically with regards to bone loss, bone resorption, and changes in overall bone structure. These adverse effects are all seen with indicators of oxidative stress and a variation in the levels of oxidative gene expression. Once gravity is restored, however, the recovery is slow and incomplete. Despite this, few reports have investigated the correlation between oxidative damage and general modifications within the bone. In this project, we will make use of a ground-based model of simulated weightlessness (hindlimb unloading, HU) in order to observe skeletal changes in response to induced microgravity due to changes in oxidative pressures. With this model we will analyze samples at 14-day and 90-day time points following HU for the determination of acute and chronic effects, each with corresponding controls. We hypothesize that simulated microgravity will lead to skeletal adaptations including time-dependent activation of pro-oxidative processes and pro-osteoclastogenic signals related to the progression, plateau, and recovery of the bone. Microcomputed tomography techniques will be utilized to measure skeletal changes in response to HU. With the results of this study, we hope to further the understanding of skeletal affects as a result of long-duration weightlessness and develop countermeasures to combat bone loss in spaceflight and osteoporosis on Earth.

Introduction

- Space travel shows decrements in the skeletal system.
- Hindlimb unloading (HU) is a rodent model used to simulate weightlessness on Earth.
- Short-term HU causes structural deficits in the bone with an increase in osteoclast responses.
- Most of the studies involving HU are done on mice, using short-duration timepoints.
- HU studies done on rats might provide a better analog to human responses to microgravity.
- It is necessary to study long-duration HU as humans want to travel to Mars.
- This study focused on the diaphyseal (midshaft) region of the rat femur, a weight-bearing bone.
- This research is part of a larger grant that will observe general effects of the skeletal system over various time periods across both genders.

Methods and Materials

- *Subjects:* Male rats at 3-months of age were housed at the University of California, Davis animal facility and were assigned into HU or control groups.
- *Hindlimb Unloading:* Rats underwent HU for 14-days or 90-days.
- *Microcomputed Tomography:* Rat femurs were used for analysis of the diaphyseal section of the bone. 3-D analysis was done at 21.8 µm resolution using the Skyscan 1272. Reconstruction was done using NRecon and GPUrecon, with a threshold at 0.0025-0.052. Rotation was done using DataViewer in order to standardize rotation of each bone for analysis. Binarization, done at a threshold of 115-255, and analysis of the bone were done in CTan.
- *Region of Interest:* In order to analyze the mid-diapysis, the total length of the bone was measured and the averaged. Once the midpoint was determined, a length of 2mm was used for analysis.



- *Figure Descriptions*: Graphs A)-E) show results from microcomputed tomography analysis of the cortical diaphysis.
- *Statistical Analysis:* Test for equal variance was performed using Levine's test. Once equal variances were confirmed, two-way ANOVA was performed. Tukey-Kramer post-hoc test was applied where interactions effects between treatment and timepoint were present. Statistical significance was set at $p \leq 0.05$.

Results

1) No significant changes were seen in the cortical parameters for 14D.
2) Substantial decrements were seen in the cortical parameters for 90D timepoints.
3) These findings show that skeletal changes are most prominent with long-duration HU.

Future Work

1) These observations will be coupled with RNA sequencing and qPCR, in order to identify relevant gene expression changes as HU progress.
2) Females will be analyzed to determine if any changes seen are sex-dependent.

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